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ORIGINAL ARTICLE

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Biological monitoring and surveillance results of Gulf War I veterans exposed to depleted uranium

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Abstract *Objective:* To relate medical surveillance outcomes to uranium biomonitoring results in a group of depleted uranium (DU)-exposed, Gulf War I veterans. *Methods*: Thirty-two veterans of Gulf War I who were victims of 'friendly fire' involving DU weapons, in whom exposure assessment can accurately be measured, had urine uranium concentrations determined using ICP-MS technology. Clinical laboratory parameters were measured and related to urine uranium concentrations. Data were examined by stratifying the cohort into a low U group, <0.10 μg/g creatinine versus a high U group, ≥ 0.10 μg/g creatinine and assessing differences between groups. *Results*: Over a decade after first exposure, soldiers possessing embedded DU fragments continue to excrete elevated concentrations of uranium in urine. No clinically significant uranium related health effects were observed in blood count, blood chemistries including renal markers, neuropsychological measures, and semen quality or genotoxicity measures. Markers of early changes in renal glomerular and tubular function were not statistically different between groups; however, genotoxicity measures continue to show subtle, mixed results. *Conclusion*: Persistent urine uranium elevations continue to be observed more than 12 years since first exposure. Despite this, renal and other clinical abnormalities were not observed, likely due to the 'relatively' low uranium burden in this cohort compared to historical uranium-exposed occupational groups. Continuing surveillance is indicated, however, due to the on-going nature of the exposure. These results are an important finding in light of the on-going controversy regarding health effects observed in soldiers of the Gulf War and other conflicts, whose uranium exposure assessment is unable to be accurately determined.

Keywords Depleted uranium · Clinical evaluation · Renal toxicity · Genotoxicity · Urine uranium · HPRT

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Introduction

The depleted uranium (DU) follow-up surveillance program dates from 1993 when the Department of Veterans Affairs initiated an effort to track the health status of Gulf War I veterans involved in friendly fire incidents with DU weaponry. Since that time, the DU Program has prospectively followed 70 veterans of the estimated 100 who are survivors of the incidents, in which approximately 20 US vehicles (Abrams tanks and Bradley fighting vehicles) were mistakenly fired upon by other US forces.

In addition to suffering traumatic injuries (burns, fractures, traumatic amputations), soldiers were also exposed to DU. The majority of these exposure incidences were of short duration and involved inhalation of aerosolized DU particles primarily in the form of uranium oxide (AEPI 1995; Parkhurst et al. 2003). These involved both service personnel on or in a hit vehicle and rescuers immediately on the scene. Suspended DU oxides may have also contaminated wounds or could have been ingested following coughing to clear airways. The unique exposure scenario that occurred that has resulted in the most significant surveillance results to date involves the service personnel who sustained shrapnel injuries. In such cases, embedded DU metal fragments in soft tissue have chronically, over time, oxidized in situ, allowing on-going DU release that is being manifested as elevated urinary uranium excretion over a long duration of time (now greater than 12 years).

The surgical management of these DU fragments has been determined by a Department of Defense protocol that considers the number and size of fragments and potential surgical morbidity related to risk of damage to vital structures. Hence, about one quarter of the 70-member cohort still possess retained metal fragments.

Although the health effects of primary concern to the public are attributed to its radioactivity, DU decays primarily by high energy emission of alpha particles, which are poorly penetrating; thus, the principal radiologic hazard from the DU is to tissues in immediate contact with internalized DU particles or fragments. The dose is ultimately a function of contact time, particle solubility and rate of elimination (Army Environmental Policy Institute 1995; Eckerman 1988).

Despite its radioactivity, uranium does not appear to be highly carcinogenic. There is poor evidence for an excess cancer risk, specifically of lung, bone, or kidney (the most likely targets) in occupational cohorts summarized in recent publications (ATSDR 1999; Institute of Medicine 2000) whose exposure intensities were greater and of longer duration than the Gulf War-exposed group. The lung cancer excess observed in uranium miners has been well documented to be attributed to radon present in the mines (Samet et al. 1989; Samet 1989). Radon is a more intensely radioactive element than natural uranium by a factor of 10,000 (Kathren and Moore 1986; Kathren et al. 1989). Little to no decay products beyond ^{234}U exist in DU, as these are separated in the processing of the uranium ore. Post ^{234}U decay products have not had sufficient time to form since leaving the uranium processing plants due to the 10,000-year half-life of thorium-230, the initial decay product of ^{234}U (Papastefanou 2002). Radiation dose estimates for Gulf War veterans with shrapnel calculated from whole-body radiation counting using the ICRP 30 Biokinetic model for uranium yielded upper limits of 0.1 rem/year (the public dose limit) and 5.3 rem/50 year (with the annual occupational exposure limit being 5 rem/year as a comparison) (McDiarmid et al. 2000).

Therefore, uranium's chemical toxicity rather than its radiological toxicity has been the primary focus of the surveillance of the Gulf War veterans with emphasis on the target organs most likely affected by uranium and other heavy metals— the kidney, the central nervous system, and the reproductive system. To date, five rounds of surveillance (1994, 1997, 1999, 2001, and 2003) have been conducted on an inpatient basis at the Baltimore Veterans Affairs Medical Center (BVAMC). This paper reports results of the 2003 clinical assessment of this cohort, 12 years since exposure first began during Gulf War I.

Materials and methods

Medical surveillance is offered bi-annually to all Gulf War I soldiers involved in DU-friendly fire incidents in 1991 for whom contact information is available, approximately 90 individuals. Participation is voluntary. Thirty-two in this cohort were evaluated at the Baltimore VA Medical Center between April and July 2003. All of these participants had been seen previously on at least one other occasion.

Clinical assessment

The clinical assessment occurred over a 3-day, in-patient hospital visit and included a detailed medical history, an extensive exposure history, a thorough physical examination, and laboratory studies. The laboratory battery included hematologic and blood clinical chemistry measures, as well as neuroendocrine and genotoxicological parameters. Semen quality was also evaluated. Spot and 24-h urine samples were obtained for measurement of clinical chemistry parameters related to renal function and for urine uranium determinations. Urine uranium determinations were standardized per gram creatinine to correct for hydration status and renal function as is typical practice for urine metal biomonitoring studies (Karpas et al. 1998; McDiarmid et al. 1999). Participants also underwent a battery of neurocognitive tests. For the 2003 DU surveillance data, we tested the association between urine uranium and three genotoxicity outcome variables: sister chromatid exchange (SCE), chromosomal aberrations (CA) and

mutation frequency (MF) at the hypoxanthine guanine phosphoribosyl transferase (HPRT) locus.

Uranium exposure assessment

Twenty-four-hour urine specimens collected during the hospital surveillance visit, were sent to the Armed Forces Institute of Pathology's (AFIP) Department of Environmental Toxicologic Pathology (Washington DC, USA) for quantitative and isotopic composition analysis by Inductively Coupled Plasma-Dynamic Reaction Cell-Mass Spectrometer (ICP-DRC-MS) (Ejnik et al. 2000). This new technique provided improved accuracy at lower U concentrations and the ability to reliably conduct isotopic analysis, which was not possible with the Kinetic Phosphorescence Assay (KPA) method utilized in previous years (McDiarmid et al. 2000, 2001a). Briefly, urine samples were prepared for quantification by diluting the urine by a factor of four with deionized water. An internal standard at 500 pg ^{233}U/ml (CRM 111A, New Brunswick laboratory, Argonne, IL, USA) was used in all standards and samples to correct for instrument drift and sample matrix effects. All solutions were prepared in 2% Optima grade nitric acid (Fisher Scientific). Quantification of uranium was achieved using an Elan 6100 DRC (Perkin-Elmer, Norwalk, CT, USA) ICP/MS by monitoring the m/z at 233.04 for $^{233}U^{+}$ and at 238.02 for $^{238}U^{+}$. The isotopic composition of uranium was determined by monitoring the m/z at 235.02 for $^{235}U^{+}$ and 238.02 for $^{238}U^{+}$. Mass discrimination was corrected for using the standard reference material CRM 145 from New Brunswick Laboratory. Each sample was measured in triplicate, and each measurement contained five replicates of 49 sweeps. The ratio of $^{235}U/^{238}U$ was calculated from the appropriate measured m/z signals. The method detection limit of quantification was 0.1 pg U/ml. The method detection limit for determining the $^{235}U/^{238}U$ was 3.0 pg U/ml. The $^{235}U/^{238}U$ ratio of natural uranium is 0.73. Any sample with a measured ratio of 0.6 or less was considered to be positive for DU. Urine uranium concentrations were corrected on the basis of urine creatinine (cre) concentrations to account for urine dilution to obtain μg U/g cre. This exposure measure was used in data analysis as its natural metric, as its natural logarithm (ln) and as a binary variable.

Urine uranium as a binary variable

Two exposure groups, high ($n = 13$) versus low ($n = 19$), were determined based on each individual participant's 2003 urine uranium results. High exposure was defined as urine total uranium concentrations greater than or equal to 0.10 μg/g creatinine, which is the exposure cut-point utilized in previous years. While there is no generally accepted standard normal urine uranium value, we chose a value intermediate between, at the low end, several estimates of mean urine uranium concentration in non-exposed populations in the literature (0.011–0.022 μg/l) (Dang et al. 1992; Medley et al. 1994; Ting et al. 1999) and, at the high end, upper dietary limits due to natural uranium in soil and ground water (up to 0.35 μg/l) urine (ICRP 1974).

Hematologic and renal toxicity measures

Hematologic parameters, serum and urine creatinine, and serum uric acid measures were evaluated by the VA clinical laboratory using standard methodologies. Aliquots taken from urine samples for β_2-microglobulin analysis were immediately neutralized using 0.5 N NaOH and analyzed by microparticle enzyme immunoassay by Quest Diagnostics Laboratory. Total protein was measured by Baltimore VA Clinical Lab using the M-TP Microprotein assay from Beckman Coulter that uses Pyrogallol Red for detection. (Watanabe et al. 1986). All other urinary proteins were measured by the Department of Nephrology-Hypertension, University of Antwerp, (Edegem-Antwerp, Belgium). These measures of nephrotoxicity included markers of glomerular or tubular dysfunction [urine retinol binding protein (RBP), and microalbumin (mAlb)], and cytotoxicity [urine intestinal alkaline phosphatase (IAP) and *N*-acetyl-D-glucosaminidase (NAG)]. Five milliliter aliquots of urine were immediately stabilized by addition of 250 μl of stabilization buffer (1 M imidazole, 2% Triton X-100, 20 mM benzamidine, 2,000 U/ml aprotinin, 1% sodium azide, pH 7.0) and frozen at −70°C until analysis. Retinol binding protein was measured using an automated nonisotopic immunoassay based on latex particle agglutination (Bernard and Lauwerys 1983). Microalbumin (mAlb) was measured by immunochemical analysis with detection by lasernephelometry (Dade Behring, Germany); NAG activity in urine was measured colorimetrically (Boehringer Mannheim Biochemica, Germany); IAP was measured by enzyme–antigen immunoassay (EAIA) as described by Verpooten et al. (1992).

Neurocognitive/psychiatric assessment

Four impairment indices were constructed from a battery of neurocognitive tests described previously (McDiarmid et al. 2004a). Norms for these indices were established from tests given to US Marine Corps recruits, mean age 19 (range 18–28), mean education 12 years (range 12–17 years) (Reeves et al. 1995). The *n* varied by test (range 84–196). The indices represent the proportion of scores falling one standard deviation below the mean. Hence, the higher the proportion, the worse the performance. The NP index was constructed from six paper and pencil tests measuring cognitive performance. The A-IIac (accuracy: percent correct),

A-IIrt (speed: median response time for correct responses) and A-IItp (throughput: a computed score combining speed and accuracy) indices were derived from computerized Automated Neuropsychological Assessment Metrices (ANAM).

Reproductive health measures

Neuroendocrine parameters

Serum follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin, thyroid stimulating hormone (TSH), free thyroxine, and total testosterone were analyzed at the Baltimore VA clinical laboratory by enzyme immunoassay using a Beckman Coulter Access 2 Analyzer.

Semen characteristics

Evaluation of semen characteristics included: volume, sperm concentration, total sperm count, and functional parameters of sperm motility. A full description of collection, processing and measurements is presented in McDiarmid et al. 2004a. In 2003, there were similar incidences of semen dilution required for computer-assisted analysis and enzyme treatment for samples not liquefied at 30 min at 37°C as compared to prior years.

Genotoxicity measures

Chromosomal aberration and sister chromatid exchange

Peripheral blood lymphocytes were cultured for the examination of background frequencies of chromosomal aberrations (CAs) and sister chromatid exchanges (SCEs) using standard methods (Perry and Wolff 1974: Evans and O'Riordan 1975; Swierenga et al. 1991). Briefly, cells were cultured for 48 h for CAs and 72 h for SCEs. After staining, 50 cells were examined from each sample for CAs and between 11 cells/sample and 25 cells/sample were examined for SCEs. Of the 32 cases examined, we were able to report SCE results for 22, (others had inadequate culture yields) of which the majority (17) had 25 cells/sample examined.

Hypoxanthine–guanine phosphoribosyl transferase mutation assay

For hypoxanthine–guanine phosphoribosyl transferase (HPRT) mutation analysis, venous blood samples (30 ml) were obtained in heparinized vacuum tubes in Baltimore and sent at ambient temperature by overnight air-mail to the BioMosaics laboratory in Burlington, VT, USA. On receipt, blood samples were centrifuged and the mononuclear cell fractions (containing the lymphocytes) were separated, washed, counted and cryopreserved in liquid nitrogen. Samples were analyzed as described previously (McDiarmid et al. 2004a), within approximately 2 weeks. The ratio of cloning efficiency (CE) in the presence of 6-thioguanine to the CE in the absence of 6-thioguanine selection defined the mutation frequency (MF).

Statistical data analysis

Tests of differences in high versus low uranium groups

For each outcome, differences in outcome measures of distribution location (e.g., median) between high and low urine uranium groups were examined using the Mann–Whitney *U* test (Wilcoxon rank sum test), which assumes equally shaped distributions (Woolson 1987), although they can differ in their means. Statistical Products and Service Solutions 12.0 (SPSS 2003) was used for these tests. To test the assumption of equally shaped distributions we used the two-sample version of the Kolmorogov–Smirnov test to compare the shapes of the distributions. In none of the comparisons did we detect significantly unequal distribution shapes. Hence, we used the Mann–Whitney exact test for all comparisons of high versus low uranium groups. While we report the results of the Mann–Whitney Test, we also generated nonparametric correlations (Spearman Rho) on all variables by the continuous urine uranium variable, but it was found to be no more sensitive than the Mann–Whitney for detecting differences

Further analysis

Associations between urine uranium and neurocognitive evaluation Because of the possible presence of outliers, robust regression, which down-weights outliers, was used to study the association between each neurocognitive index and the natural logarithm (ln) of the continuous urinary uranium variable. Because any nonlinearity of continuous urinary uranium in the linear regression model for the neurocognitive indices could produce apparent outliers, we also studied the association using fractional polynomial transformations of ln continuous urinary uranium variable. Appropriate analyses were done to study associations when adjusting for confounders. Robust regression and fractional polynomial transformations were done using STATA 2003 (StataCorp 2003).

Associations between urine uranium and MF We studied the association between the ln urine uranium and ln MF using fractional polynomial transformations. We tested the relative contributions of covariates to the fit by the process of backward elimination to determine whether any covariate would become significant. We also removed outliers to determine their influence on the fit.

Probability levels

Because this is a surveillance program, it is important to attend to and follow even subtle differences between high and low uranium groups. However, because the number of DU-exposed Gulf War Veterans in this group is small ($n = 32$), the power to detect subtle effects is low. To deal with this problem we attend to differences that would ordinarily be dismissed as having occurred by chance (probability of 0.2 or less, two-tailed test). We do this if the differences are in line with biological plausibility, in the expected direction, or are consistent with previous findings. In addition, we report and explore differences that meet the standard criteria of statistical significance (probability of 0.05 or less, two-tailed test).

Results

The demographic characteristics of the 32 members of this cohort are presented in Table 1 along with a comparison to the whole group ($n = 70$) of Gulf War veterans examined at least one time. Seven of these participants are still on active duty in the US Army. As can be seen from the table, the veterans participating in the surveillance program in 2003 were very similar to the group as a whole.

Biologic monitoring for uranium

The results of the 24-h total urine uranium analysis for the 2003 cohort are presented in Fig. 1. Uranium concentrations for this group ranged from 0.001 μg/g creatinine to 41.8 μg/g creatinine. All values equal to or over 0.1 μg/g creatinine were from participants with known retained shrapnel fragments and U isotopic analysis results indicated that presence of DU in their urine samples. Three individuals with shrapnel injuries had urine U concentrations below 0.1 μg /g creatinine and no evidence of DU in their urine, suggesting that their shrapnel was ordinary metal fragments. Isotopic analysis for DU was positive in one veteran known to have had shrapnel but with low urine U concentrations (< 0.1 μg U/g creatinine) which is consistent with his history of some shrapnel removal.

Clinical findings

As reported in the past (McDiarmid et al. 2000, 2001a), there were no clinically significant differences in laboratory parameters between the low and high uranium groups. Only one statistical difference was observed in serum phosphorus concentrations (3.75 mg/dl vs. 4.11 mg/dl, respectively; $P < 0.03$ Mann–Whitney exact test) (Table 2). However, both of these values fall within the normal clinical range for serum phosphorus.

Hematologic parameters

Means for red cell and platelet measures for both the high and low uranium groups were within normal clinical limits and were not significantly different between the two groups. There were also no significant differences in any parameters of the differential counts or percentages (data not shown).

Hepatic function

Liver transaminases (SGOT and SGPT) were also within normal limits and did not differ between high and low uranium groups (data not shown).

Table 1 Demographic characteristics of the DU Follow-up Program participants

	All participants ($n = 70$)		2003 participants ($n = 32$)	
	n	%	*n*	%
Race				
African American	22	31.4	10	31
Caucasian	38	54.3	18	56
Hispanic	7	10	2	6
Other	3	4.2	2	6
Education				
High school graduate	21	30	6	19
Some college	36	51.4	21	66
College graduate (4-year degree)	8	11.4	3	9
Post baccalaureate	4	5.7	2	6
Unknown	1	1.4		
Marital status[a]				
Never married	10	14.3	4	13
Married	49	70	25	78
Divorced	10	14.3	3	9
Unknown	1	1.4		
Age[b]	38.2 ± 4.91		38.5 ± 1.01	

[a]Marital status as of participant's most recent visit
[b]Age as of 12/31/2004 ± SD

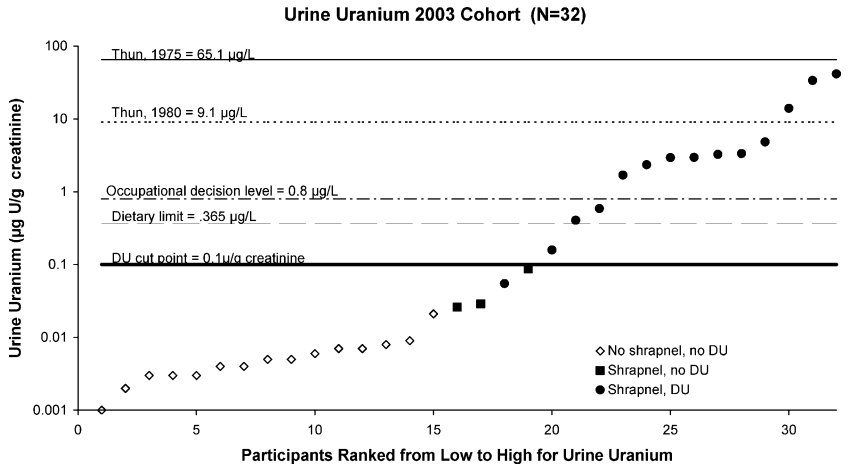

Fig. 1 Twenty-four hour total urine uranium analysis for DU-exposed veterans of GWI from the 2003 evaluation. The top two lines (65.1 and 9.1 μg/l) represent the mean total urine uranium found in a sub-cohort of uranium fabrication workers in 1975 and 1980, as reported in a study by Thun. The alternating dot/dash line (0.8 μg/l) depicts an occupational exposure decision level used at the Department of Energy's Fernald Environmental Management Project (McDiarmid et al. 2000; Fernald Environmental Management Project 1997) as a trigger for investigating work areas for sources of elevated uranium exposure. The dotted line (0.365 μg/l) is an upper limit for the dietary contribution of uranium in urine for a general population from drinking water (ICRP 1974; McDiarmid et al. 2000). This value was calculated by dividing the upper limit for 24-h uranium excretion for "reference man" by 1.4 L/24 h. Studies have shown that corrections per gram creatinine and per liter urine are generally equal for "reference man" and for this group of veterans with normal renal function (Ting 1999; NHANES 2003). The *bold solid line* (0.01 μg/g creatinine) indicates the cut point established by the DU Follow-up Program to identify low versus high urine uranium concentrations (McDiarmid et al. 2000)

Renal function parameters

Test results measuring renal function from the 2003 surveillance visit (Table 2) showed no statistically significant ($P < 0.05$) differences between the low and high urine uranium groups, except for serum phosphate concentrations as noted above. Urine excretion of phosphate was not different, however, suggesting that the elevated serum level was not a result of impaired renal function. The mean values for all other urine and serum parameters fell within their normal clinical ranges. The mean retinol binding protein in urine concentration was higher in the high urine uranium group compared to the low urine uranium group, as would be predicted by an inhibitory effect of uranium on protein reabsorption by renal proximal tubule cells. Statistically, however, the increase was not significant due to the wide range of the results in the high uranium group.

Neurocognitive evaluation

Responses assessed by the neurocognitive indices were within normal ranges regardless of whether respondents were in the high or low uranium group. Consistent with

Table 2 Renal parameters

Laboratory test (normal range)	Low uranium group[a] [mean ± SE (*n*)][c]	High uranium group[b] [mean ± SE (*n*)]	Mann–Whitney test (*P*)
Urine creatinine (1.3–2.6 g/24 h)	1.82 ± 0.15 (18)	1.98 ± 0.15 (13)	0.31
Urine calcium (100–300 mg/24 h)	180.96 ± 19.26 (18)	194.62 ± 20.07 (12)	0.79
Urine PO_4 (0.4–1.3 g/24 h)	0.92 ± 0.10 (17)	1.04 ± 0.15 (12)	0.44
Urine β_2 microglobulin (0–160 μg/g creatinine)	63.33 ± 11.86 (12)	74.36 ± 13.28 (11)	0.53
Urine intestinal alkaline phosphatase (IAP) (< 2.0 U/g creatinine)	0.35 ± 0.08 (19)	0.33 ± 0.15 (13)	0.85
Urine *N*-acetyl-ß-glucosaminidase (NAG) (< 5 U/g creatinine)	1.27 ± 0.17 (19)	0.99 ± 0.14 (13)	0.38
Urine total protein (1–150 mg/24 h)	64.81 ± 8.8 (16)	51.14 ± 11.86 (12)	0.21
Urine micro-albumin (< 25 mg/g creatinine)	9.29 ± 4.13 (19)	4.17 ± 1.21 (13)	0.85
Urine retinol binding protein (< 610 μg/g creatinine)	27.33 ± 3.08 (19)	80.51 ± 51.35 (12)	0.54
Serum creatinine (0.0–1.4 mg/dl)	1.03 ± 0.04 (19)	0.92 ± 0.03 (13)	0.11
Serum calcium (8.4–10.2 mg/dl)	9.28 ± 0.09 (19)	9.25 ± 0.07 (13)	0.85
Serum PO_4 (2.7–4.5 mg/dl)	3.75 ± 0.11 (19)	4.11 ± 0.12 (13)	0.03
Serum uric acid (3.4–7.0 mg/dl)	5.77 ± 0.25 (19)	5.56 ± 0.45 (13)	0.32

[a] < 0.10 μg/g creatinine
[b] ≥ 0.10 μg/g creatinine
[c] *n* number of participants' samples analyzed. Difference in "*n*" due to laboratory errors in sample processing

Table 3 Semen characteristics

Laboratory test (normal range)	Low uranium group[a] (mean ± SE)	High uranium group[b] (mean ± SE)	Mann–Whitney test (*P*)
Days abstinence (2–5)	3.6 ± 0.4	2.9 ± 0.4	0.223
Semen volume (2–5 ml)	2.6 ± 0.4	2.7 ± 0.4	0.973
Sperm concentration (> 20 million/ml)	153.2 ± 54.0	228.4 ± 49.1	0.173
Total sperm count (> 40 million)	358.7 ± 94.1	540.9 ± 117.1	0.282
Percent motile sperm (> 50%)	64.7 ± 3.3	68.5 ± 5.4	0.197
Total progressive sperm (> 20 million)	107.4 ± 31.5	189.3 ± 43.8	0.152
Percent progressive sperm (> 50%)	27.5 ± 3.3	31.6 ± 2.8	0.426
Total rapid progressive sperm (> 10 million)	70.6 ± 20.8	136.7 ± 33.3	0.114
Percent rapid progressive sperm (> 25%)	17.8 ± 3.0	22.6 ± 2.8	0.223

[a] < 0.10 μg/g creatinine ($n = 11$) [b] > 0.10 μg/g creatinine ($n = 10$)

previous years, there were no statistically significant differences in any of the neurocognitive indices between the high and low uranium groups. However, using the "low bar" probability level of 0.2 or less, those in the high uranium group had a higher impairment score on accuracy (A-IIac—derived from the computerized battery) than those in the low uranium group using the Mann–Whitney exact test ($P = 0.1$). The fractional polynomial regression procedure revealed a significant cubic association between urine uranium and the accuracy index (A-IIac) ($P = 0.04$), adjusting for general intellectual level, age and emotional status. Of these, only general intellectual level was significantly associated with accuracy ($P = 0.005$). On inspection of the data, it was clear that the relationship between urine uranium and the accuracy impairment index for this test was being driven by two cases with extremely high uranium values who also have persistent complications due to combat injuries such as amputations or post-traumatic stress disorder.

Reproductive health measures

Neuroendocrine function

No statistically significant differences were observed in mean prolactin, FSH, LH, testosterone, free thyroxine or TSH values and all results were generally within the normal clinical range (data not shown).

Semen characteristics

Semen samples were obtained from a total of 28 subjects in the 2003 cohort. Seven of these subjects were azospermic, six of whom were in the low urine uranium group. Five of these seven cases had been vasectomized; two of the seven subjects were azospermic for unknown reasons, one each in the high and low urine uranium groups. Data from these azospermic subjects (days abstinence and liquefaction status) were excluded from data analysis. For the remaining 21 subjects, the distributions of abstinence period, semen volume, and incidence of incomplete liquefaction were not significantly different between exposure groups (data not shown). The incidence of subnormal sperm count and motility characteristics (below WHO 1987 norms) were not significantly different between low and high uranium exposure groups (data not shown). Means of semen characteristics for subjects with high urinary uranium were generally greater than subjects in the low urinary uranium group (Table 3), however, none of these differences were statistically significant ($P > 0.05$).

Genotoxicity

Sister chromatid exchange and chromosomal aberration

No difference in SCE between the high and low urine uranium groups was found using the Mann–Whitney exact test. SCE for the low and high uranium groups were 5.05 ± 0.25 and 4.73 ± 0.34, respectively, $P = 0.441$. Results are not reported from this visit from the chromosomal aberrations analysis since all values but one of the 32 analyzed were zero, making statistical analysis of the CA data inappropriate.

Hypoxanthine–guanine phosphoribosyl transferase mutation assay

The Mann–Whitney exact test was used to examine differences in mutation frequency (MF) by low (< 0.1 μg urine uranium/g creatinine) vs. high (≥0.1 μg urine uranium/g creatinine) levels. Although mean MF was higher in the high uranium group (15.91 ± 1.96 vs. 32.38 ± 13.75), the difference between the two groups was not significant ($P = 0.82$). An analysis of the association between ln MF and ln urine uranium was done using fractional polynomial transformations of log of the continuous urine uranium. That association had a *P* value of 0.067 (Fig. 2). However, the pattern was similar to, but weaker than, the association reported in 2001. We did similar analyses in which all covariates (blood cloning efficiency, current smoking, recent X-rays, age) were entered and with backward stepping were removed one at a time. None were significant. Removing the two largest outliers, one above and one

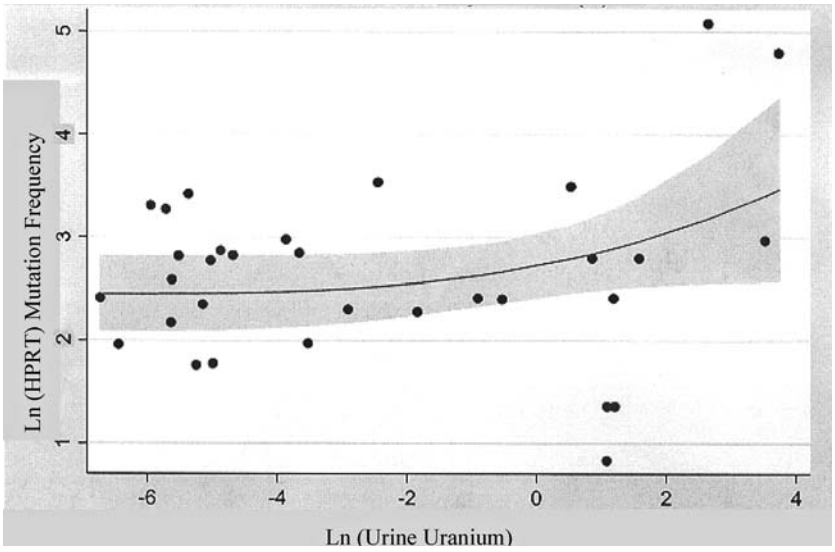

Fig. 2 Fractional polynomial regression of ln (HPRT) on ln (urine uranium)

below the curve, flattens the curve slightly and increases the *P* value to 0.12.

Discussion

Medical surveillance continues to be offered to all surviving members of several "friendly fire" incidents involving DU weaponry during the first Gulf War. Of the estimated 100 survivors, contact information is known for about 90, and 70 of the 90 have been evaluated at least one time. While the size of this cohort is small, our ability to measure urine uranium, the exposure parameter on which health risk is determined, enlarges the importance of the surveillance of this group. In addition, our opportunity to follow this group prospectively adds value to the observations about health impact. As well, from previous work with this cohort and from results of more than 900 Gulf War and Operation Iraqi Freedom soldiers who submitted urine for uranium testing, we know this subcohort of Gulf War soldiers with retained metal fragments possess the highest urine uranium values we have seen in any cohort tested (McDiarmid et al. 2001b, 2004b; M.A. McDiarmid, unpublished). The clear determinant of high urine uranium concentration has been the presence of retained uranium-containing metal fragments in soft tissue in this and all of our previous evaluations (Hooper et al. 1998; McDiarmid et al. 2000, 2001a, 2004a). For those soldiers possessing metal fragments, the size of these depots is sufficiently large to sustain ongoing elevations in urine uranium measures as the fragments oxidize in situ. For the majority who do not have retained metal fragments, but were exposed to DU through inhalation or wound contamination, any initial systemic uranium has been eliminated or transported to longer-term storage sites such as bone. Consequently, their uranium burden is in a steady state, with minimal release from body stores as evidenced by their low urinary uranium excretion.

Clinical evaluations

Over the years, there has been a clear absence of a specific "signature" laboratory finding or medical problem shared by this cohort. As well, there have been no clinically significant differences in any laboratory parameters between high and low uranium groups. There have also been few statistically significant differences, save the persistent urine uranium elevation in the sub-group of the cohort with retained metal fragments. Multiple outcomes were examined and thus there exists the risk that statistically significant findings may be observed by chance alone. In this most recent survey performed in 2003, even the occasional statistical differences between low and high urine uranium groups were almost absent. We therefore chose to focus only on the outcomes of highest interest in this paper.

Renal function parameters

Although the kidney is the putative 'critical' organ for uranium toxicity under acute and chronic exposure conditions (Gilman et al. 1998; Leggett 1989; Zamora et al. 1998), we observed no evidence of clinical renal dysfunction (glomerular or tubular) in this 2003 cohort. The biomarkers for proximal tubule dysfunction, the presumed target of uranium (Leggett 1989), showed minimal differences between the high and low urine uranium groups. The statistically significant difference in total urinary protein that was higher in the high uranium group seen previously (McDiarmid et al. 2004a) was not present in the 2003 cohort. However, results from the current evaluation were similar to those found in 2001 for urinary retinol binding protein (RBP), a more spe-

cific marker of proximal tubule function. A 1.5-fold elevation in the RBP urine concentration mean value for the high uranium group approached statistical significance in 2001, while in this 2003 surveillance visit a 2.5-fold higher mean value was observed, but it was not statistically significant. Because kidney concentrations of uranium have been shown to increase with time under chronic exposure conditions (Pellmar et al. 1999; Squibb et al. 2001), evidence of small changes in renal proximal tubule function may be sentinels for early effects being observed. We therefore enlarged the effects monitoring to include more detailed characterization of glomerular and tubular function and injury (urinary uric acid, calcium, phosphate, microalbumin, urinary β_2-microglobulin, retinol binding protein, *N*-acetyl-β-glucosaminidase, and intestinal alkaline phosphatase). Although results from these did not show marked differences based on uranium exposure, the need for continued surveillance of renal function in this exposed cohort is clear.

Neurocognitive evaluation

In general, there were no significant differences in neurocognitive functioning between high and low uranium groups. A significant association noted in the A-IIac index was based on the test performances of two individuals who had both extremely high uranium values in conjunction with other clinical confounders. Similar results were observed in the 2001 data (McDiarmid 2004a, b). There is still insufficient evidence to suggest that uranium exposure leads to impaired functioning; however, we will continue surveillance of neurocognitive performance in the future.

Reproductive health measures

Neuroendocrine function

Neuroendocrine and thyroid measures were not different between the two uranium groups and were within normal limits with the exception of serum prolactin, which demonstrates an elevated level outside the normal range in both the low and high uranium groups. Although other metal exposed populations have experienced neuroendocrine effects (Cullen 1984; Gustafson 1989), making these endpoints biologically plausible targets of uranium toxicity, the multiple observations concern arises. The experience over time is also helpful here, given that in previous evaluations, other prolactin relationships were observed with a mild elevation in prolactin in the high uranium group (McDiarmid et al. 2000) and most recently in the low uranium group (McDiarmid et al. 2004a). No difference in the prolactin levels, however, were observed in an intervening year (McDiarmid et al. 2001a). Results from future evaluations may provide some clarity as to effects taking place.

Semen characteristics

For the parameters evaluated in this study, both uranium exposure groups have normal semen characteristics based on average values. Since semen characteristics do not have an upper limit for normality, the generally elevated values in the high uranium exposure group are not considered clinically significant for an individual's fertility.

Genotoxicity

Definitive differences between groups in genotoxicity measures have not been observed over the last four surveillance visits. In two of these previous surveillance rounds, no differences in CAs were seen between the high and low uranium groups; although there was a statistically significant increase in SCE observed in the high uranium group in one of these previous evaluations (McDiarmid et al. 2001a), but not observed before that time (McDiarmid et al. 2000). Against this mixed picture, we again report from this most recent surveillance no difference in SCE baseline frequency. We observed similar results in the 2001 surveillance (McDiarmid et al. 2004a). HPRT mutation frequencies (MFs), performed for the first time in 2001 and again in this surveillance, were higher in both visits in the high uranium groups, and the difference was statistically significant in 2001 even when adjusted for smoking, age and frequency of X-rays. The MF difference between the high and low U groups was not significant in this 2003 visit; however, a similar fractional polynomial association was observed. In comparing results between surveillance visits, it is important to recognize that the patient cohorts do not contain exactly the same individuals from year to year. Trends in repeated results from the same individuals will be more fully explored in a future paper on longitudinal analysis. The tendency towards increased HPRT MFs and the variable results observed in the other genotoxicity markers suggest a need to continue to closely monitor genotoxicity in succeeding visits.

Only one other uranium-exposed human cohort has been examined for genotoxic endpoints (uranium fuel production and enrichment workers) and findings reported were an increase in SCEs, total CAs and dicentrics as a function of uranium exposure (Martin et al.1991). Two cell culture experiments have documented uranium's genotoxicity. Studies in Chinese hamster ovary (CHO) cells exposed to uranyl nitrate (UO_2^{2+}) found an increased frequency of micronuclei, SCE and CAs (Lin et al. 1993). In a human osteoblast (HOS) cell line, increased SCE and an increase in transformation to a tumorigenic phenotype was seen in DU exposed cells in culture (Miller et al. 1998a). Miller et al. (1998b) also showed increased urine mutagenicity in TA98 and AMES II (TA 7001–7006) in DU-implanted animals. More recently, this group has reported genetic instability in the HOS cell line after DU exposure manifested as

delayed lethality and micronuclei formation (Miller 2003).

Genetic instability has been raised as a proposed mechanism for genotoxic insult from previous exposure by a number of investigators studying other cohorts exposed to putative chemical or radiological genotoxicants (Au et al. 1996). This theory holds that previous genotoxic exposure predisposes cells to hyper-express an effect when subsequently exposed to a provocative genotoxic challenge. Related to this hypothesis in a radiologic exposure scenario is the phenomenon of a ''by-stander effect.'' Here, the usual ''all or none'' phenomenon of cell death or sparing from radiologic exposure, allows for an intermediate effect of cell alteration of contiguous neighboring (by-stander) cells. These cells are just on the border of radiation energy penetration. This theory allows the apparent lack of radiologic intensity and poorly penetrating effect to still play a potential role in genotoxicity observed—rather than assuming any effect is chemically induced. To examine more carefully the role of radiological versus chemical effects in the DU-exposed cohort, HPRT mutant isolates were recovered for molecular analysis of their mutational changes to determine if the spectrum of mutations in DU exposed individuals differ from expectations based on background in vivo spectra in humans and spectra for low and high LET ionizing radiations and chemicals (Albertini 2001). These studies are on-going.

Conclusions

In two additional years of observation (12 years since first exposure) in a cohort of GWI veterans for whom exposure to DU can be documented, persistence of urine uranium elevations is observed. This documents an ongoing uranium exposure from soft tissue DU depots in steady state and suggests vigilance in continued surveillance of this cohort. Lack of renal effects, despite the kidney's reputation as the 'critical' organ, is likely due to the 'relatively' low uranium burden in this cohort compared to highly exposed historical occupational groups. However, biologically plausible measures of renal tubular function and structural integrity have yielded results suggestive of, though not statistically significant for, an early effect from DU exposure. Genotoxicity endpoints continue to reveal mixed results and require further follow-up. The subtle, but biologically plausible findings in this chronically exposed, sentinel cohort continue to reassure on the one hand, but recommend ongoing surveillance on the other. Continued observation ensures early detection of any yet to develop outcomes in this exposed cohort and informs predictions of future effects in other potentially exposed populations.

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References

Army Environmental Policy Institute (AEPI) (1995) Health and environmental consequences of depleted uranium use in the US army Atlanta, GA

Albertini RJ (2001) HPRT mutation in humans: biomarkers for mechanistic studies. Mutat Res 489:1–16

Albertini RJ, Hayes RB (1997) Somatic mutations in cancer epidemiology. In: Toniolo P, Boffetta P, Shuker D, Rothman N, Hulka B, Pearce N, (eds) IARC Scientific Publication No 142:159–184

ATSDR. Toxicological Profile for Uranium (update) (1999) US Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry

Au WW, Wilkinson GS, Tyning SK, Legator MS, Hallberg L (1996) Monitoring populations for DNA repair deficiency and for cancer susceptibility. Environ Health Perspect 104:579–582

Beck AT, Steer RA, Brown GK (1996) Manual for the beck depression inventory. The Psychological Corporation, San Antonio

Bernard A, Lauwerys R (1983) Continuous flow system for automation of latex immunoassay by particle counting. Clin Chem 29:1007–1011

Cole J, Skopek TR (1994) International commission for protection against environmental mutagens and carcinogens, working paper 3: somatic mutant frequency, mutation rates and mutational spectra in the human population in vivo. Mutat Res 304:33–106

Cullen M, Kayne R, Robins J (1984) Endocrine and reproductive dysfunction in men associated with occupational inorganic lead intoxication. Arch Environ Health 39:431–440

Dang HS, Pullat VR, Pillai KC (1992) Determining the normal concentration of uranium in urine and application of the data to its biokinetics. Health Phys 62:562–566

Eckerman K (1988) Limiting values of radionuclide intake and air concentration and dose conversion factors for inhalation submersion and ingestion Federal Guidance Report No 11. EPA, Washington, DC

Ejnik EJ, Carmichael AJ, Hamilton M, McDiarmid M, Squibb K, Boyd P, Tardiff W (2000) Determination of the isotopic composition or uranium in urine by inductively coupled plasma mass spectrometry. Health Phys 78:143–146

Evans HJ, O'Riordan ML (1975) Human peripheral blood lymphocytes for the analysis of chromosome aberrations in mutagen tests. Mutat Res 31:135–148

Fernald Environmental Management Project (1997) Technical basis for internal dosimetry at the fernald environmental management project, 1997. 2008.97. A.D. Fernald, OH, FEMP

Gilman AP, Villeneuve DC, Secours VE, Yaagminas AP, Tracy BL, Quinn JM, Valli VE, Moss MA (1998) Uranyl nitrate: 91-day toxicity studies in the New Zealand white rabbit. Toxicol Sci 41:129–137

Gustafson A, Hedner P, Schutz A, Skerfving S (1989) Occupational lead exposure and pituitary function. Int Arch Occup Environ Health 61:281

Hooper FJ, Squibb KS, Siegel EL, McPhaul K, Keogh JP (1998) Elevated urine uranium excretion by soldiers with retained uranium shrapnel. Health Phys 77:512–519

ICRP (1974) Report of the task groups on reference man, vol 23. Elmsford, NY, Pergamon Press

Institute of Medicine (2000) Gulf War and Health, vol 1. In: Fulco CE, Liverman CT, Sox HC (eds) National Academy Press, Washington DC

Kane RL, Reeves DL (1997) Computerized test batteries. In: Horton A, (ed) The neuropsychology handbook: new edition. Springer, New York, Berlin, Heidelberg

Karpas Z, Kirber A, Eliah E, Marcus P, Roiz Y, Marko R, Kol R, Brikner D, Halicz L (1998) Uranium in urine—normalization to creatinine. Health Phys 74:86–90
Kathren RL, Moore RH (1986) Acute accidental inhalation of U: a 38-year follow-up. Health Phys 51:609–619
Kathren RL, McInroy JF, Moore RH, Dietert SE (1989) Uranium in the tissues of an occupationally exposed individual. Health Phys 57:17–21
Kim JP, D'Arpa P, Jacobson-Kram D, Williams JR (1985) Ultraviolet-light exposure induces a heritable sensitivity to the induction of SCE by mitomycin-C. Mutat Res 149:437–442
Leggett RW (1989) The behavior and chemical toxicity of U in the kidney: a reassessment. Health Phys 57:365–383
Lin RH, Wu LJ, Lee CH, Lin-Shiau SY (1993) Cytogenetic toxicity of uranyl nitrate in Chinese hamster ovary cells. Mutat Res 319:197–203
Lundgren K, Lucier GW (1985) Differential enhancement of sister-chromatid exchange frequencies by alpha-naphthoflavone in cultured lymphocytes from smokers and non-smokers. Mutat Res 143:131–135
Martin F, Earl R, Tawn EJ (1991) A cytogenetic study of men occupationally exposed to uranium. Br J Ind Med 48:98–102
McDiarmid MA, Hooper FJ, Squibb K, McPhaul K (1999) The utility of spot collection for urinary uranium determination in depletd uranium exposed Gulf War veterans. Health Phys 77:261–264
McDiarmid MA, Keogh JP, Hooper FJ, McPhaul K, Squibb KS, Kane R, DiPino R, Kabat M, Kaup B, Anderson L, Hoover D, Brown L, Hamilton M, Jacobson-Kram D, Burrows B, Walsh M (2000) Health effects of depleted uranium on exposed Gulf War veterans. Environ Res 82:168–180
McDiarmid MA, Engelhardt SM, Oliver M (2001b) Urinary uranium concentrations in an enlarged Gulf War veteran cohort. Health Phys 80:270–273
McDiarmid MA, Squibb K, Engelhardt S, Oliver M, Gucer P, Wilson PD, Kane R, Kabat M, Kaup B, Anderson L, Hoover D, Brown L, Jacobson-Kram D (2001a) Surveillance of depleted uranium exposed Gulf War veterans: health effects observed in an enlarged "friendly fire" cohort. J Occup Environ Med 43:991–1000
McDiarmid MA, Engelhardt SM, Oliver M, Gucer P, Wilson PD, Kane R, Kabat M, Kaup B, Anderson L, Hoover D, Brown L, Handwerger B, Albertini RJ, Jacobson-Kram D, Thorne CD, Squibb KS (2004a) Health effects of depleted uranium on exposed Gulf War veterans: a 10-year follow-up. J Toxicol Environ Health, Part A 67:277–296
McDiarmid MA, Squibb K, Engelhardt SM (2004b) Biologic monitoring for urinary uranium in Gulf War I veterans. Health Phys 87(1):51–56
Medley DW, Kathren RL, Miller AG (1994) Diurnal urinary volume and uranium output in uranium workers and unexposed controls. Health Phys 67:122–130
Miller AC, Blakely WF, Livengood D, Whittaker T, Xu J, Ejnik JW, Hamilton MM, Parlette E, John TS, Gerstenberg HM, Hsu H (1998a) Transformation of human osteoblast cells to the tumorigenic phenotype by depleted uranium-uranyl chloride. Environ Health Perspect 106:465–471
Miller AC, Fuciarelli AF, Jackson WE, Ejnik EJ, Emond C, Strocko S, Hogan J, Page N, Pellmar T (1998b) Urinary and serum mutagenicity studies with rats implanted with depleted uranium or tantalum pellets. Mutagenesis 13:643–648
Miller AC, Brooks K, Stewart M, Anderson B, Shi L, McLain D, Page L (2003) Genomic instability in humanosteoblast cells after exposure to depleted uranium: delayed lethality and micronuclei formation. J Environ Radiol 64:247–259
National Health and Nutrition Examination Survey (NHANES) (2003) Second National report on human exposure to environmental chemicals. NCEH Publication No 02-0716. Atlanta, GA: Centers for Disease Control and Prevention
O'Neill JP, McGinniss MJ, Berman JK, Sullivan LM, Nicklas JA, Albertini RJ (1987) Refinement of a T-lymphocyte cloning assay to quantify the in vivo thioguanine-resistant mutant frequency in humans. Mutagenesis 2:87–94
O'Neill JP, Sullivan JP, Booker JK, Pornelos BS, Falta MT, Greene CJ, Albertini RJ (1989) Longitudinal study of the in vivo hprt mutant frequency in human T-lymphocytes as determined by a cell cloning assay. Environ Mol Mutagen 13:289–293
Papastefanou C (2002) Depleted uranium in military conflicts and the impact on the environment. Health Phys 83:280–282
Parkhurst MA, Szrom F, Guilmette RA, Holmes TD, Cheng YS, Kenoyer JL, Collins JW, Sanderson TE, Fliszar RW, Gold K, Beckman JC, Long JA (2003) Capstone depleted uranium aerosols: Generation and characterization, vol 2, Appendix D. Prepared for the US Army by Pacific Northwest National Laboratory under a Related Services Agreement with the US Dept of Energy, PNNL-14168
Pellmar TC, Fuciarelli AF, Ejnik JW, Emond C, Mottaz HM, Landauer MR (1999) Distribution of uranium in rats implanted with depleted uranium pellets. Toxicol Sci 49:29–39
Perry P, Wolff S (1974) New Giemsa method for the differential staining of sister chromatids. Nature 251:156–158
Reeves DL, Levinson D, Batsinger K, Winger B, Gastaldo E (1995) ANAM-USMC normative data. Report No:NCRF-SR-95-05. National Cognitive Recovery Foundation, San Diego
Robinson D, Goodall K, Albertini RJ, O'Neill JP, Finette B, Sala-Trepat M, Tates AD, Beare D, Green MHL, Cole J (1994) An analysis of in vivo HPRT mutant frequency in circulating T lymphocytes in the normal human population: a comparison of four databases. Mutat Res 313:227–247
Samet JM (1989) Radon and lung cancer. J Natl Cancer Inst 81:745–757
Samet JM, Pathak DR, Morgan MV, Marbury MC, Key CR, Valdivia AA (1989) Radon progeny exposure and lung cancer risk in New Mexico U miners: a case-control study. Health Phys 56:415–421
Squibb KS, Leggett RW, McDiarmid MA (2001) Predicting long term kidney effects of depleted uranium (DU) exposure in Gulf War vets with embedded DU shrapnel. Toxicol 60:359
StataCorp. Stata Statistical Software. [8.0] (2003) College Station, TX, Stata
Statistical Products and Service Solutions. SPSS Statistical Software. [12.0] (2003) Chicago, SPSS
Swierenga SH, Heddle JA, Sigal EA, Gilman JP, Brillinger RL, Douglas GR, Nestmann ER (1991) Recommended protocols based on a survey of current practice in genotoxicity testing laboratories, IV Chromosome aberration and sister-chromatid exchange in Chinese hamster ovary, V79 Chinese hamster lung and human lymphocyte cultures. Mutat Res 246:301–322
Ting BG, Paschal DC, Jarrett JM, Pirkle JL, Jackson RJ, Sampson EJ, Miller DT, Caudill SP (1999) Uranium and thorium in urine of United States residents: reference range concentrations. Environ Res 81:45–51
Verpooten GF, Nuyts GD, Hoylaerts MF, Nouwen EJ, Vassanyiova Z, Dlhopolcek P, Debroe ME (1992) Immunoassay in urine of a specific marker for proximal tubular S3-segment. Clin Chem 38:642–647
Watanabe N, Kamei S, Ohkubo A, Yamanaka N, Ohsawa S, Makino K, Tokuda K (1986) Urinary protein as measured with a pyrogallol red-molybdate complex, manually and in a Hitachi 726 automated analyzer. Clin Chem 32:1551–1554
WHO (1987) WHO laboratory manual for the examination of human semen and semen-cervical mucus interaction. Cambridge University Press, Cambridge
Woolson RF (1987) Statistical methods for the analysis of biomedical data. Wiley, New York
Zamora ML, Tracy BL, Zielinski JM, Meyerhof DP, Moss MA (1998) Chronic ingestion of uranium in drinking water: a study of kidney bioeffects in humans. Toxicol Sci 43:68–77